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Controlling continuous high cell density perfusion culture with the Alternating Tangential Flow system in real time using radio-frequency impendance

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Monitoring and controlling high cell density perfusion culture with the Alternating Tangential Flow (ATF) system in real time using radio-frequency impedance

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PRINCIPLE BEHIND ABER BIO-CAPACITANCE (BC)

BC measurement is based on the principle of dielectric spectroscopy, whereby an infinitesimal, alternating electrical field is generated by an Aber Futura probe, which polarises live cells with an intact membrane, which is impermeable to charge (dielectric), thereby restricting the free flow of ions. This allows a capacitive charge to build up around the cell membrane. The Aber probe is capable of simultaneously emitting the electrical field, and detecting this capacitive charge build up. The capacitance signal generated is proportional to the live bio-volume of the cells present. Unlike other methods of biomass measurement, BC does not measure dead cells, gas bubbles or debris. BC probes are now available in diameters of 7.5mm (PICO), 12mm and 25mm, as well as single use options (see Figure 1).

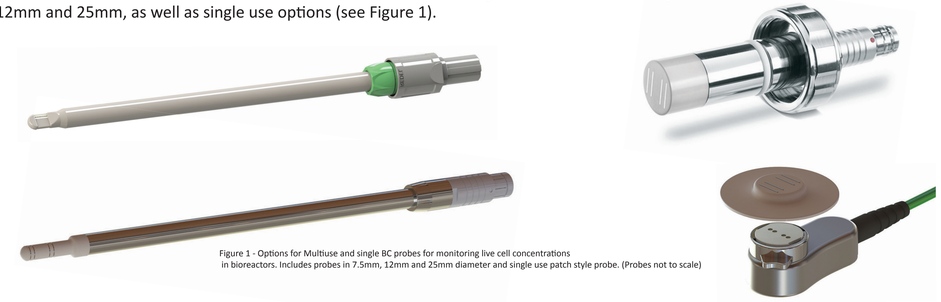

Figure 1 - Options for Multiuse and single BC probes for monitoring live cell concentrations in bioreactors. Includes probes in 7.5mm, 12mm and 25mm diameter and single use patch style probe. (Probes not to scale)

BIO-CAPACITANCE BASED FEEDBACK CONTROL

In many cell culture manufacturing processes it is traditional to define a fixed-volume feed strategy for nutrient feeds, based on historical cell demand. However, one major drawback of this strategy is that once the feed volumes are defined, they are inflexible to batch-to-batch variations in cell growth and physiology. This can lead to inconsistent productivity and product quality. An auto feedback system using online Aber BC measurements makes it possible to automatically control the complex feed rates (Zhang *et al*., 2015). The nutrient feed amount can be determined by integrated biomass, (IBC), which is calculated with Aber BC by determining the area under the BC curve across a predetermined time interval (typically 24 h).

The original approach in the study by Zhang *et al*. (2015) was to convert the BC reading to VCD and then feed based on the estimated cumulative of estimated cell growth (cICG). However, the BC reading was found to directly correlate with the cICG-based feed amount which made it feasible to directly use the online BC reading to control the feed rate. This also eliminates the need to use models based on either the Cole-Cole equation-based approach or multivariate analysis (Lee *et al*. , 2014; Carvell *et al*., 2017) to adjust capacitance to match the offline viable cell density (VCD) during the later stages of cell growth, where there may be a divergence observed between the measurements. When looking at the online capacitance and offline measurements closely, there could be a strong argument that the BC measurement provides a signal that is more closely related to the metabolic activity of the cells (Braasch *et al*., 2013).

PERFUSION MONITORING AND CONTROL

Many cGMP cell culture processes are based on a perfusion process. Control of the feed or addition rates to maintain pseudo-steady-state conditions in these bioreactors can be especially challenging due to high and fluctuating cell concentrations that can rapidly change environmental conditions. With infrequent manual daily sampling based on trypan blue exclusion haemocytomer cell counting, the control system can have too little information on which to base an appropriate decision to manipulate the process, and hence will lead to large process deviations. Tight control of the perfusion or concentrate addition rate allows the bioreactor to be operated under the optimum conditions for maximum recombinant protein production.

A robust automatic perfusion rate control system based on BC has also been successfully used in cell culture manufacturing processes (Carvell & Dowd, 2006). The system operates in a completely closed loop i.e. no samples need to be taken to obtain process information, thus mitigating contamination risks. In the control algorithm, a cell specific perfusion rate is specified and the BC signal is converted into a perfusion flow rate through calculation and implementation with a variable speed controlled pump. To demonstrate a perfusion control rate system, we have used data provided by the FDA (Division of Biotechnology Review and Research-II OBP/OPQ/CDER) with bioreactors run in the batch, fed batch and perfusion modes. In the perfusion mode the bioreactor was equipped with an XCell ATF device (Repligen) and a capacitance probe (Aber Instruments Ltd, UK). A photograph of the experimental set up is shown in Figure 2.

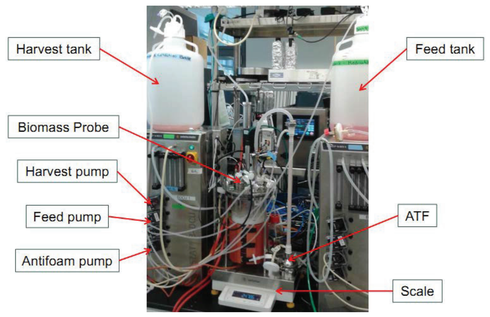

Figure 2 - Perfusion set up at FDA using the XCell ATF and Aber capacitance probe.

In Figure 3, the viable cell densities for cultures run in the three different modes are compared between the Nova Bioprofile method (based on image analysis and Trypan blue) and the real time BC values. In the perfusion mode the capacitance probe was shown to immediately spot a sudden increase in live cell density after 12 days of culture caused by too much media being pumped out in error. This underlies an additional advantage of using a capacitance probe to measure the VCD so that errors or sudden failures can be picked up immediately.

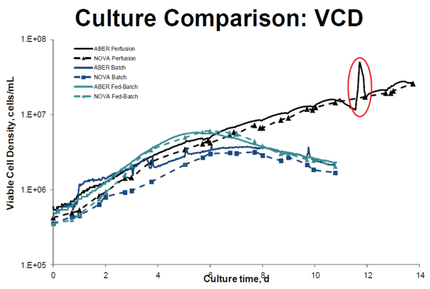

Figure 3 - Viable cell density versus time for batch, fed batch and perfusion modes. The BC values are based on converting the capacitance at 580 KHz to a viable cell density.

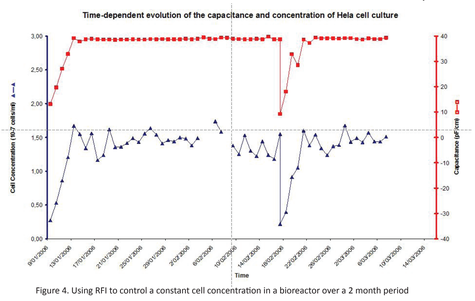

Figure 4. Using RFI to control a constant cell concentration in a bioreactor over a 2 month period

The RFI probe only measures the viable cell mass and is therefore ideal for this application and it has been applied for process control in sono-perfused cytostats, spin-filter perfused bioreactors and for maintaining steady-state, continuous culture of bioreactors with external loop filters (Figure 2 Repligen ATF system) for monoclonal antibody and recombinant protein production.

An example of the actual time-dependent capacitance trace of a perfused Hela cell culture evolving from batch (preset volume, increasing concentration) to fedbatch (increasing volume, preset cell concentration) growth conditions is shown in Figure 4. The stable capacitance value can be seen when the culture is operated in a fedbatch mode with a preset cell concentration of 10^7 cells/ml. The peak observed during this step represents an increased cell concentration due to an insufficient fresh medium supply to maintain a stable capacitance.

FUTURA PICO

The Aber biomass system is now a vital part of many advanced bioreactor system and the probes are commonly used for controlling critical feed rates or for maintaining constant cell concentrations in perfused systems. With the technology based on capacitance also being available in a single use format, the Aber probe is a critical component in many new cell processes that are scaled up into cGMP production. And now with the introduction of the Futura PICO, users will be able to use the robust and trusted capacitance measurements at a much smaller scale, as well as allowing users to introduce PAT to evaluate critical parameters in to their process far earlier than ever possible before, supporting the acquisition of key data to ultimately expedite time to market and return on investment for leading Biopharmas.

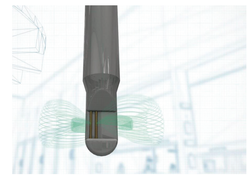

Figure 5a. Visualisation of the PICO probe's electrical field. The PICO emits an electrical field with its unique, patented, trapezoidal electrode design layout.

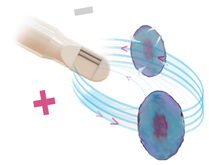

Figure 5b. Visualisation of the PICO probe's electrical field interacting with and polarising live cells.

The new Futura PICO system launched at BPI Boston in 2017 has a unique, patented 7.5 mm diameter probe that can be autoclaved or steam sterilized in situ. The four pure platinum electrodes are beneficially positioned to maximize the current path between the electrodes around the tip of the probe body and produces a smaller symmetric field of measurement compared to other capacitance based probes, while having a comparable performance to other probe configurations in the Aber range (Fig. 6a & b).

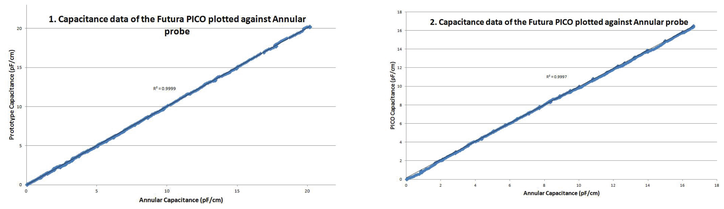

Figure 6a & 6b. Comparative plot of the capacitance values of the Futura PICO against an Annular probe during the step change study. R2 values of 0.9999 & 0.9997 indicate a highly significant correlation.

The PICO probe displaces a minimal culture volume and can be placed just a few millimetres away from the side wall and other parts of the vessel making it ideal for use in bioreactors of 500ml and less. The PICO probe connects to the smallest and lightest available headamplifier on the market and utilizes the latest generation of capacitance based electronics from Aber. The PICO system is set up so that the capacitance readings from the live biomass will be the same as generated by the Futura systems with the larger annular or flush style probes. The small footprint and compact electrode configuration make the PICO probe ideal for use in wide range of mini-bioreactor systems. Applications are already being opened up for using the probe in 3D Cell culture or in mini vessels with volumes less than 20ml. Aber has the ability to produce customized probes so if you need a specific configuration then contact one of our specialists in either USA or UK.

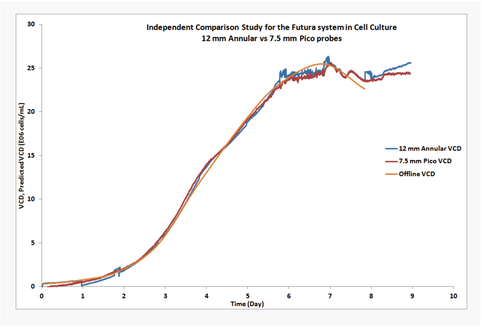

Figure 7 showing an independent Futura system comparison study between the 12 mm Annular probe and the newly launched 7.5 mm Pico for measuring a cell culture process. Both probes were placed in a Dasgip Benchtop reactor. As can be seen from the figure, the performance of both probes was highly comparable and the cell concentration trends followed each other nicely. In addition, both probes correlated very well with offline viable cell density measurement.

CONCLUDING REMARKS

This poster summarises the use of the Aber capacitance technology as an advanced process analytical tool across various scales. The auto-feedback control strategy based upon Bio Capacitance feedback control demonstrated remarkable enhancement in process robustness over traditional fixed fed-batch processes.
The feed amount was automatically adjusted in response to process variations in real time to avoid over-/under-feeding due to seed density variation. This strategy may be used as part of a toolbox for developing the next generation of cell culture manufacturing processes that can take advantage of automated, real-time control of nutrient levels.

The ability of the real time Aber measurement to detect a process deviation accurately in a perfusion process was also discussed. This can prove to be invaluable, especially when used in manufacturing, where errors or deviations can be spotted reliably so appropriate action(s) can be taken.

The final example in this poster shows how two different designs of a RFI probe perform when used in to measure a batch cell culture. Figure 6 shows a comparison study between the 12 mm Annular probe and the newly launched 7.5 mm Pico for measuring a CHO cell culture process. Both probes were placed in a Dasgip benchtop reactor. The performance of both probes was highly comparable and the cell concentration trends followed each other nicely. In addition, both probes correlated well with offline viable cell density measurement. At the time of writing, the 7.5 mm PICO probe is the smallest diameter reusable capacitance sensor available in the market. The performance of the small probes is particularly important if small bioreactors are being used in scale up studies and it is important to have the same pF/cell biovolume for an individual scale for the small design compared with the probe designs used in production cGMP vessels. Not only can the capacitance probes be used to measure cell concentration in different scale up platforms, but the capacitance trends obtained can be used to determine the success of a scale up process or strategy.

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